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First report of *Pseudoveronaea ellipsoidea* causing sooty blotch and flyspeck in China

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Abstract — *Pseudoveronaea ellipsoidea* is reported and described as a newly recorded species from China. This fungus was isolated from peels of hawthorn and apple fruit collected from Shaanxi and Shanxi Provinces, and caused sooty blotch and flyspeck signs on these hosts. Based on morphological characteristics and phylogenetic analysis, we identified our isolates as *P. ellipsoidea.* This is also the first report of the genus *Pseudoveronaea* in China.

Key words — *Dissoconiaceae,* taxonomy, morphology, phylogeny

Introduction

Sooty blotch and flyspeck (SBFS) fungi colonize the waxy cuticle of a wide range of plant hosts in humid regions worldwide. They cause cosmetic damage to cultivated fruits and ornamental plants due to the smudges formed on the surface, resulting in economic losses to growers (Williamson & Sutton 2000, Batzer et al. 2005, Gleason et al. 2011). For nearly 75 years this disease was considered to be two different diseases, each caused by a single species (Colby 1920, Williamson & Sutton 2000). Based on analysis of rDNA sequences combined with traditional morphology, researchers obtained a better understanding of SBFS, the number of species in the complex increased to more than 80 (Díaz Arias et al. 2010; Frank et al. 2010; Gleason et al. 2011; Li et al. 2011, 2012), and many new genera and species were described in the discovery process.

Among these fungi, *Pseudoveronaea* was recently established as a new genus and named after its morphological similarity to *Veronaea* (Li et al.

2012). A member of the family *Dissoconiaceae*, *Pseudoveronaea* is nevertheless phylogenetically and morphologically distinct from other genera in this family, in that it produces only one type of conidia and these are septate. Currently there are only two species in this genus, *P. ellipsoidea* and *P. obclavata*, both associated with SBFS on apple (Gleason et al. 2011, Li et al. 2012).

In our recent study on SBFS, four isolates obtained from hawthorn and apple were identified as *P. ellipsoidea,* based on morphological comparison and phylogenetic analysis of the ITS region.

Materials & methods

Isolates and morphology

Hawthorn (*Crataegus pinnatifida* Bunge) and apple (*Malus domestica* Baumg.) fruit with SBFS signs were collected from Shaanxi and Shanxi Provinces in 2011. Peels with SBFS were cut off, examined and photographed under the dissecting microscope, then pressed between paper towels until dry and preserved. Pieces of SBFS colonies on the peels were transferred to potato-dextrose agar (PDA) and cultured at 25°C in the dark (Sun et al. 2003). After one month, pure isolates were obtained and transferred to synthetic nutrient-poor agar (SNA) for morphological examination and description using the method of Zhang et al. (2009). Colony descriptions were based on cultures on PDA and oatmeal agar (OA) after one month in the dark at 25°C. Specimens of representative dried cultures were deposited in the Fungal Herbarium of Northwest A&F University, Yangling, Shaanxi Province, China (HMUABO).

DNA extraction, PCR and sequencing

Genomic DNA was extracted from fungal mycelium according to the protocol of Barnes et al. (2001). The primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used to amplify the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. The PCR conditions followed the methods of Zhuang et al. (2010). PCR products were sequenced by Sangon Biotech (Shanghai), China.

Sequence alignment and phylogenetic analysis

Sequences in this study and other sequences downloaded from GenBank were put into CLUSTAL X to perform preliminary alignment (Thompson et al. 1997), and then manually adjusted in BioEdit v. 5.0.9.1 (Hall 1999). Maximum parsimony (MP) analysis of aligned sequences was conducted using PAUP v. 4.0b10 (Swofford 2003). Heuristic searches were performed with a 1000 random taxa addition and tree bisection-reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and given equal weight and gaps were treated as missing data. The robustness of clades and internal branches was evaluated by 1000 bootstrap replications. Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC) were also calculated. Sequences generated in this study were deposited in GenBank. Alignment and the representative tree were deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S13736).

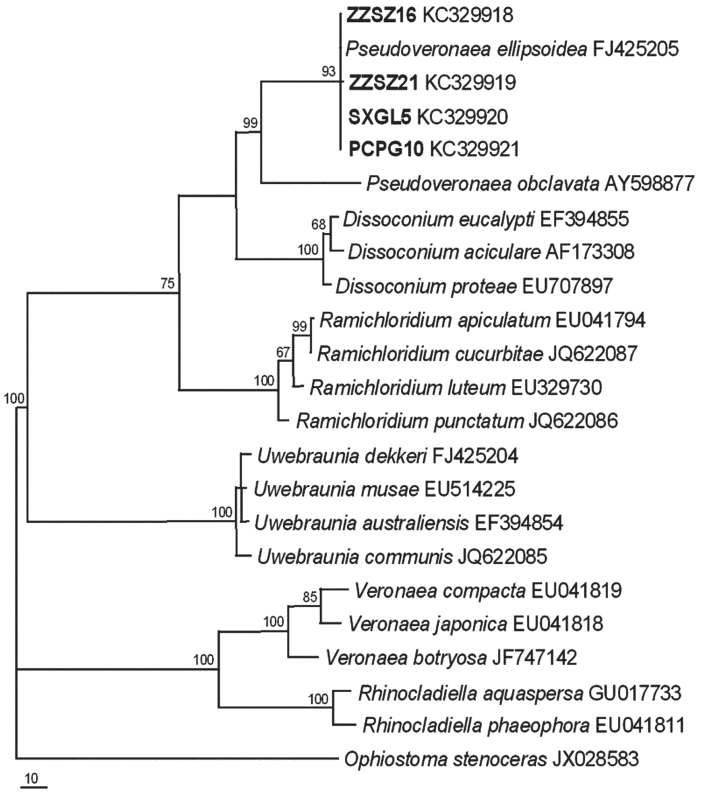

Fig. 1. One of four equally most parsimonious trees obtained from a heuristic search with 1000 random taxon additions of the ITS sequence alignment. The bootstrap support values (>50%) based on 1000 replicates are shown at the nodes. The tree was rooted to *Ophiostoma stenoceras*. The scale bar shows 10 changes. Strains investigated in this paper are presented in bold.

Results

Phylogenetic analysis

The manually adjusted ITS alignment contained 23 sequences (including the outgroup sequence) and 604 characters including alignment gaps. Of these characters, 223 were parsimony-informative, 102 were variable and parsimony-uninformative, and 279 were constant. Four equally most parsimonious trees were obtained from the analysis, the first of which is shown in Fig. 1 (TL = 601 steps, CI = 0.7920, RI = 0.8808, RC = 0.6976). Two major clades were resolved from the analysis. One clade included five species in *Veronaea* and

Rhinocladiella. The other clade, with 100% bootstrap support, contained 17 strains in *Pseudoveronaea, Dissoconium, Ramichloridium,* and *Uwebraunia*. Our strains clustered together with *P. ellipsoidea* in the first subclade with a bootstrap value of 93%, indicating that they might represent the same species.

Taxonomy

Pseudoveronaea ellipsoidea Batzer & Crous, Persoonia 28: 119, 2012 Fig. 2

Hyphae septate, branched, smooth, hyaline and turning to brown as culture aged, 2–3.5 µm diam. Conidiophores erect, solitary, arising from aerial hyphae, straight to flexuous, unbranched, 3–11-septate, subcylindrical, smooth, thick-walled, brown, 35–115 × 3–4.5 µm. Conidiogenous cells terminal, integrated, subcylindrical, with apical taper to acutely rounded apex, pale brown to brown, 6–21 × 3–5 µm, sympodially proliferating, rachis straight or flexuous, scars slightly darkened and unthickened, 0.5–1 µm diam. Conidia solitary, 0–1-septate, ellipsoid to obclavate, hyaline to pale brown, (6–)8–11 (–16) × 3–4 µm, base truncate, slightly darkened and thickened, 1–1.5 µm diam.

Cultural characteristics — Colonies after 1 month on PDA at 25°C flat, raised at the middle, slightly folded, with lobate, smooth margin and sparse aerial mycelium, surface iron-gray, reverse black, reaching (18–)20(–23) mm diam. On OA faster growing, flat, spreading, with lobate, feathery margin and moderate aerial mycelium, surface olivaceous gray, reverse black, reaching 32 mm diam.

On peels of hawthorn fruit, showing the ridged honeycomb mycelial type characterized by clumps and ridges of mycelia, olivaceous gray, without distinct edges. On peels of apple fruit, forming the fuliginous mycelial type characterized by uniform mats of mycelia with distinct, feathered edges.

Specimens examined: **CHINA, Shaanxi Province**: Xi'an City, Zhouzhi County, on fruit surface of hawthorn (*Crataegus pinnatifida*), 15 Oct. 2011, Chen C & Dang JL ZZSZ16 (HMUABO; GenBank KC329918), ZZSZ21 (HMUABO; GenBank KC329919); Weinan City, Pucheng County, on fruit surface of apple (*Malus domestica*), 25 Sept. 2011, Li WH & Gao L PCPG10 (HMUABO; GenBank KC329921); **Shanxi Province**: on fruit surface of apple (*Malus domestica*), 16 Oct. 2011, Li WH & Gao L SXGL5 (HMUABO; GenBank KC329920).

Discussion

Based on phylogenetic analysis and morphological characteristics, we identified our isolates as *P. ellipsoidea*, a newly recorded species for China. This is also the first time that the genus *Pseudoveronaea* has been reported from China.

Previous study showed *P. ellipsoidea* causing SBFS on apple as displaying the fuliginous mycelial type (Li et al. 2012). In our study, *P. ellipsoidea* formed the

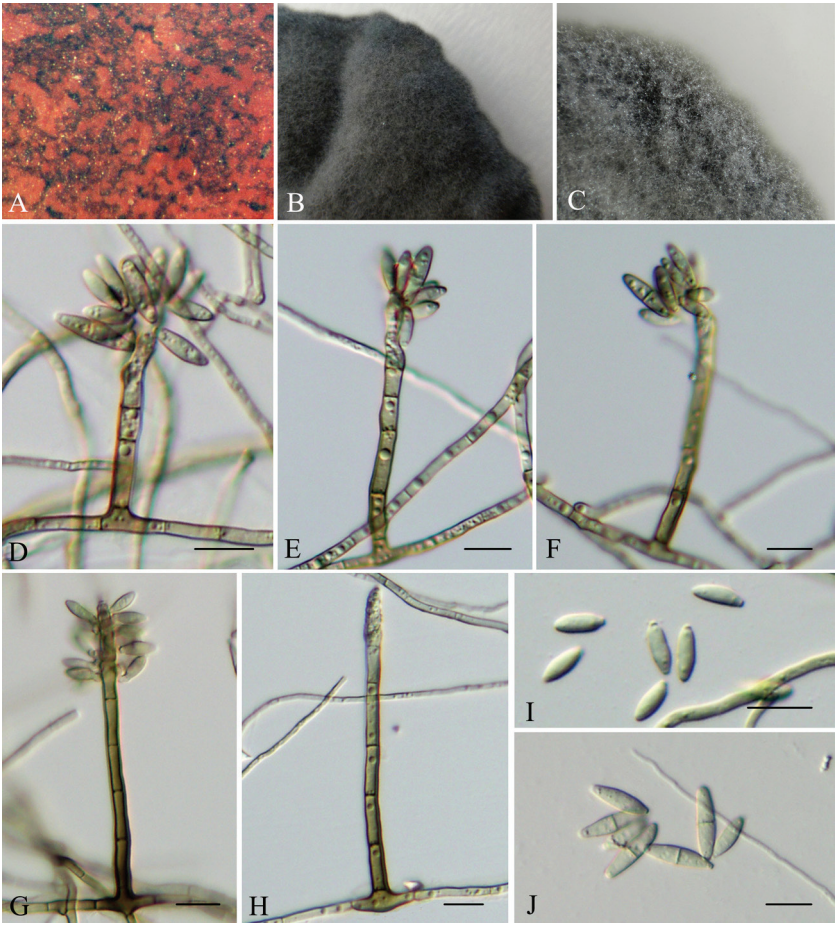

Fig. 2. *Pseudoveronaea ellipsoidea* (ZZSZ16). A. Signs on hawthorn. B. Colony on PDA. C. Colony on OA. D–H. Conidiophores showing rachis, scars and conidia. I–J. Conidia. Bars: D–J = 10 µm.

same mycelial type on apple, but caused a different type, ridged honeycomb, on hawthorn. Hemnani et al. (2008) also found a similar phenomenon in that the same SBFS species produced different mycelial types on different hosts. More investigations are needed to clarify the relationships between hosts and SBFS mycelial types.

So far, only two *Pseudoveronaea* species have been reported in USA (Li et al. 2012) and China. More collections and reports on the genus are required to help us understand the taxonomy, biogeography, and ecology of this genus.

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